The SRF/NKX 2.5 Regulatory Node

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ABSTRACT

Background: Serum Response Factor, SRF, is a regulatory protein that helps with the process of gene transcription. SRF binds to DNA sequences called Serum Response Elements (SRE). A model of SRF and NKX 2.5 was constructed to understand the feasibility and scope of my proposed project. The x-ray crystal structures of the proteins used for SRF and NKX 2.5 were 1HBX and 3RKQ, respectively. SRF was bound on the opposite side of the DNA relative to NKX 2.5 based on our sequence alignment. This configuration makes the preliminary model plausible.

Methods: The two proteins was loaded into the program PyMol: "fetch 1hbx" and "fetch 3rkq" chain W from 1HBX will be aligned with chain C from 3RKQ and their residues will be from -4 to -3 and 2 to 8, respectively. Then aligned based on the selected residues for each chain, so the subsequent command will be: "align 1hbx_resid_-4-3, 3rkq_resid_2-8."

Results: The model showed NKX 2.5 and SRF were trans to each other meaning SRF was on the opposite side of DNA from NKX 2.5. It was seen that two strands from NKX 2.5 and SRF went down the same groove which may lead to a steric clash or could potentially lead to conformational change.

Conclusion: Transcription regulation by direct and mutated TF could spawn a new class of therapeutic agents. The action of SRF can be nudged toward proliferation or conversely nudged toward differentiation. Both effects could have a significant impact on wound healing as well as heart repair.

BACKGROUND

SRF, serum response factor, functions as a regulatory protein directing gene transcription via interactions with cofactors and other transcription factors. SRF binds to DNA sequences, called Serum Response Elements (SRE). In order for SRE to be activated, it needs binding of a ternary complex factor (TCF) and SRF to specific DNA sites. SRF and ELK-1 TCF are able to bind through B-Box and SRF-MADS interactions. SRE works alongside of the cardiogenic homeodomain factor NKX2.5 which helps SRF to function in order to initiate transcriptional activation energy of the cardiac alpha-actin promoter. The cardiac alpha-actin SRE3 in human, as well as mouse, is: TTCCTTACATGGTC.

OBJECTIVE

I hypothesize that SRF and NKX 2.5 are binding a common locus to regulate gene expression. My aim is to structurally align SRF and NKX2.5 on SRE DNA sequences to explore possible ways cells can be regulated to drive proliferation vs differentiation.

METHODS

Structure alignments were completed using the tool PyMol and developing scripts that select specific DNA base pairs from each crystal structure; using one as the template and the other to move to that target. This allows structure function relationships to be inspected and evaluated relative to the sequence preferences that these TF are known to bind to. Finding synergistic binding modes among SRF and other regulatory TF's could elucidate novel mechanisms of dual regulation.

The align sequence used:
The NKX 2.5 Sequence:

```
5'                    3'
TGAAGTGyyyyyCACTTGA
ACTTCACyyyyyGTGAACT
```

As stated before, the best SRE is SRE-3 whose YY1 consensus is: **AA**N**ATGG**N**C/G**

When SRF is aligned with NKX 2.5:

```
5'              ********** 3'
CACACCGGAAGTCCTAATTAGGCCAT        SRF
           TGAAGTGyyyyyCACTTGA    NKX2.5
     TATGTCTATGCAAATAAGTG         SRE3-SRF-M
```

This alignment shows that there is room for the residues to up or down 1 or 2 forming different potential complexes.

For SRF, chain W and X are used from 1HBX for the alignment:

```
                ^633
CACACCGGAAGTCCTAATTAGGCCAT   chain W,X
TGTGGCCTTCAGGATTAATCCGGTAG
```

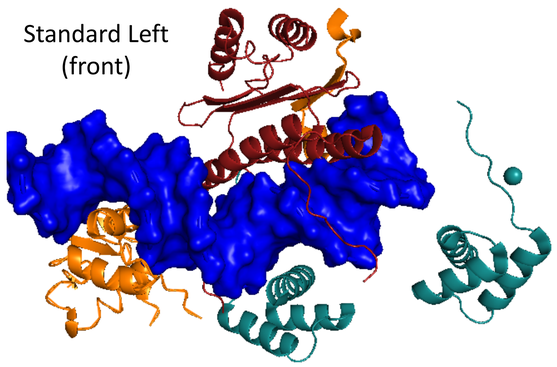
For NKX 2.5, chain C and F are used from 3RKQ for the alignment:

```
GATGGCCTAATTAGGACTTCCGGTGT   chain C,F
```

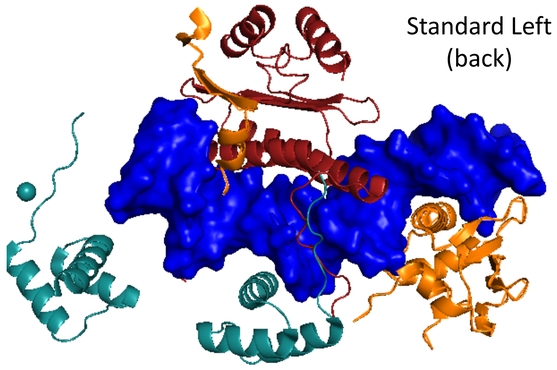
RESULTS

MY DEVELOPED MODELS

Standard Left (front)



Standard Left (back)



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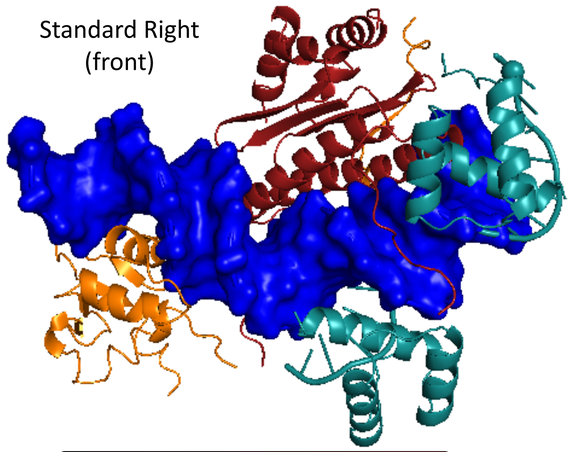
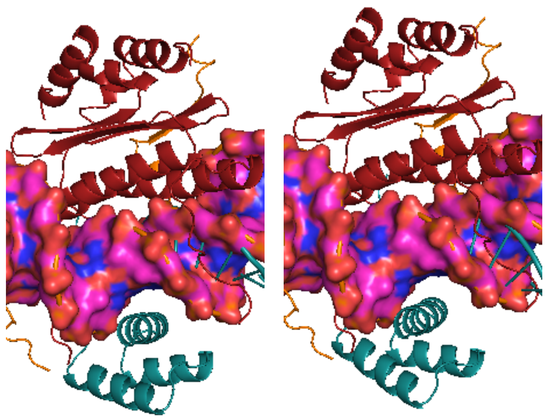
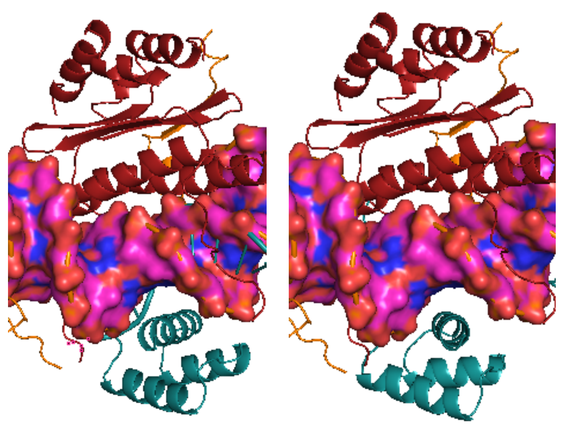


DOWN LEFT SHIFTS



Down Shift 1 | Down Shift 3

UP LEFT SHIFTS



Up Shift 1 Frame 2 | Up Shift 3 Frame 2

SRF and NKX 2.5 make contact with DNA in two different places. Cofactors increase its efficiency of binding. NKX 2.5 might cobind some DNA locus and change the affinity of SRF, so it is a much better binder, and it increases efficiency by a thousand-fold. SRE works alongside of the cardiogenic homeodomain factor Nkx-2.5 which helps SRF to function in order to initiate transcriptional activation energy of the cardiac α-actin promoter. The cardiac α-actin SRE3 in human, as well as mouse, is: (-203) TTCCTT**AC**A**TGG**T**C** (-190). However, the YY1 consensus is **AA**N**ATGG**N**C/G**. Another coaccessory transfactor that functions with SRF is GATA4 which aids with SRF myogenic genes when activating transcription. The SRF and GATA4 are able to interact when SREs obtain LIM protein CRP2 to facilitate their interaction with the MADS αI coil. This then goes on to compete with the ETS factors which are interacting with the MADS αII coil. Therefore, this cofactor is able to help with the enhancement of SRF transactivation activity. ETS Factor (similar to ELK-1 and SAP-1) finds an adjacent ETS bonding site next to the SRF site. Now, ETS factor stabilizes the SRF complex even if the SRF is mutated, and it drives SRF gene activity, but only replication. SRF complexed with ETS factor activates a subset of SRF targets. While it is essentially inhibiting an alternate set by the fact that it cannot compete YYY1 off (issue).

CONCLUSIONS

SRF and NKX 2.5 could bind at a common locus in order to help with the regulation of gene expression. By investigating how SRF interacts with SRE and other cofactors, we can implement it into the study of mechanisms and strategies to help with the improvement of heart function. Enhancing SRF's binding to ELK-1 increases cell proliferation; whereas, if we improve SRF cobinding with NKX 2.5, we might facilitate differentiation.

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